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Cigarette smoking and *KRAS* oncogene mutations in sporadic colorectal cancer: Results from the Netherlands Cohort Study

M.P. Weijenberg [a,*], P.W.M. Aardening [a], T.M. de Kok [b], A.F.P.M. de Goeij [a], P.A. van den Brandt [a]

[a] *GROW-School for Oncology and Developmental Biology, University Maastricht, Department of Epidemiology, Maastricht University, Maastricht, The Netherlands*
[b] *Nutrition and Toxicology Research Institute Maastricht (NUTRIM), Department of Health Risk Analysis and Toxicology, Maastricht University, Maastricht, The Netherlands*

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Abstract

Since a *KRAS* oncogene mutation is an early event in colorectal cancer development and cigarette smoking is thought to have an effect on early stages of colorectal tumorigenesis, smoking, especially long-term smoking, may be associated with the risk for colorectal cancer with *KRAS* oncogene mutations.

In the Netherlands Cohort Study on diet and cancer ($n = 120{,}852$ men and women), using a case–cohort design, adjusted incidence rate ratios (RR) and 95% confidence intervals (CI) were computed for colorectal tumors with wild-type and with mutated *KRAS* gene, and with specific G:C → T:A or G:C → A:T point mutations in *KRAS*, according to cigarette smoking status, frequency, duration, pack years, age at first exposure, years since cessation, inhalation and filter usage. After 7.3 years and excluding the first 2.3 years, 648 cases and 4083 sub-cohort members were included in the analyses.

Ex-smokers, but not current smokers, were at increased risk for colorectal cancer with wild-type *KRAS* gene tumors when compared with never smokers, albeit not statistically significant (RR 1.26, 95% CI 0.96–1.66). This was not observed for *KRAS* mutated tumors when comparing ex-smokers with never smokers (RR 1.15, 95% CI 0.79–1.66). The highest category of smoking frequency (>20 cigarettes/day) and inhalation of smoke were associated with an increased risk for colorectal cancer with wild-type *KRAS* gene tumors, though not statistically significant, when compared with never smoking (frequency: RR 1.24, 95% CI 0.90–1.71 and inhalation: RR 1.25, 95% CI 0.94–1.67). These associations were strongest in men (ex-smokers: RR 1.79, 95% CI 1.00–3.20; frequency: RR 1.91, 95% CI 1.03–3.52; inhalation: RR 1.69, 95% CI 0.94–3.04). No associations were observed between any of the smoking characteristics and the risk for colorectal cancer with mutated *KRAS* gene tumors, nor where there any clear associations with tumors with specific G:C → A:T transitions or G:C → T:A transversions.

These results suggest that, in contrast to the hypothesis, smoking does not increase the risk for colorectal tumors with a mutated *KRAS* gene. Some smoking characteristics, i.e. being an ex-smoker, frequency and inhalation, may be associated with risk for colorectal cancer characterized by the wild-type *KRAS* gene, especially in men.

Keywords: Smoking; Colorectal cancer; Prospective study; Netherlands Cohort Study; *KRAS* oncogene; Molecular epidemiology

1. Introduction

With the burning of tobacco products, numerous genotoxic compounds are formed, including carcinogenic polycyclic aromatic hydrocarbons, heterocyclic amines and aromatic amines [1]. Smoking is associated with a higher risk for cancer in various tissues, including lung, oral cavity, pharynx, larynx, pancreas, urinary bladder, renal pelvis, nasal cavities and paranasal sinuses, nasopharynx, stomach, liver, kidney, cervix and esophagus [1,2]. Although associations between smoking and colorectal carcinomas have been inconsistent, long-term, heavy smoking has been associated with a two- to threefold increased risk for colorectal adenomas [3]. In addition, current smoking has been observed to be more strongly associated with

* Corresponding author. Tel.: +31 43 3882358; fax: +31 43 3884128.
E-mail address: MP.Weijenberg@epid.unimaas.nl (M.P. Weijenberg).

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hyperplastic polyps than with adenomatous polyps [4]. As most colorectal carcinomas arise from pre-existing benign neoplasms, the association between smoking and colorectal adenomas led to the assumption that smoking may be involved in early tumorigenesis. Development of colorectal cancer is thought to be a multi-step process which involves the accumulation of aberrations in a number of genes [5]. The Fearon and Vogelstein model assumes the involvement of the *KRAS* (v-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog) oncogene in the transition from intermediate adenomas to carcinomas in sporadic colorectal cancer [6]. Somatic mutations can cause oncogenic activation of *KRAS*. This genetic alteration occurs in adenomas as well as in carcinomas in colon and rectal cancer. Approximately 90% of the activating mutations are found in codons 12 and 13. The predominant mutations are G:C → A:T transitions and G:C → T:A transversions [7–10]. Because smoking is thought to be involved in the early stages of colorectal tumorigenesis, smoking may be associated with mutations in the *KRAS* oncogene. Results from rodent studies support this hypothesis: benzo(a)pyrene was found to induce G:C → T:A transversions and *N*-nitrosamines were found to induce G:C → A:T transitions in *RAS* oncogenes [11]. Both chemicals are present in tobacco smoke.

The aim of the present study is to investigate the relationship between cigarette smoking and risk for overall colorectal cancer and risk for colorectal cancer with wild-type or mutated *KRAS* oncogene tumors in a prospective cohort study. We studied associations between cigarette smoking and the risk for colon, rectosigmoid and rectal cancer, with wild-type or mutated *KRAS* oncogene tumors within the Netherlands Cohort Study on diet and cancer (NLCS). Additionally, we assessed associations between cigarette smoking and colorectal cancer with a specific G:C → T:A or G:C → A:T point mutation.

2. Materials and methods

The prospective Netherlands Cohort Study (NCLS) on diet and cancer was started in 1986. The total cohort includes 120,852 persons aged between 55 and 69 years old at baseline, of which 58,279 are men and 62,573 are women. The study subjects originated from 204 Dutch municipal population registries. In order to be included, municipalities had to satisfy two criteria: (a) availability of a computerized population registry and (b) sufficient cancer follow-up coverage. A self-administered questionnaire on diet, other risk factors for cancer and potential confounders was completed at baseline [12]. The entire cohort is being monitored for cancer occurrence by annual record linkage to the Netherlands Cancer Registry (NCR) and Pathologisch Anatomisch Landelijk Geautomatiseerd Archief (PALGA), a nationwide database of histo- and cytopathology reports [13]. Combining the NCR and PALGA resulted in almost 100% coverage of the municipalities included in the NLCS.

The present analyses were carried out over the 7.3-year period of follow-up since September 1986. Accumulation of person-time in the cohort has been estimated through biennial vital status follow-up. A sample of 5000 men and women were randomly selected from the cohort after baseline exposure measurement to estimate the number of personyears for the entire cohort, whereas cases are enumerated for the entire cohort [14,15]. The sub-cohort was followed-up biennially to assess information on vital status and migration in order to calculate accumulated person-time in the cohort. Excluded from the sub-cohort were cases with prevalent cancer other than non-melanoma skin cancer. Because of incomplete nationwide coverage of PALGA alone in some of the municipalities included in the NLCS in that period and because of possible pre-clinical disease affecting exposure status, the first 2.3 years of follow-up were also excluded. In the period from 1989 till 1994, 925 incident cases with histologically confirmed colorectal cancer were observed, of which 815 could be linked to a PALGA report of the lesion. With a PALGA report, tumor tissue form eligible colorectal cancer patients could be located and identified in Dutch pathology laboratories. Collection of tumor tissue specimens started in August 1999 and was completed in December 2001. The loss to follow-up of tissue samples of cases amounted to 5%. Colorectal cancer was classified according to site as follows: colon, i.e. cecum through sigmoid colon (ICD-O-1 codes: 153.0, 153.1, 153.2, 153.3, 153.4, 153.5, 153.6, 153.7, 153.8, 153.9), rectosigmoid (ICD-O-1 code 154.0) and rectum (ICD-O-1 code 154.1).

After approval by the Medical Ethical Committees (MEC) of Maastricht University, PALGA and the NCR, tumor material was collected. All pathology laboratories in the Netherlands agreed to make relevant tissue samples available for this study. The 815 tissue samples were distributed among 54 pathology laboratories throughout the Netherlands. Only 44 (5%) tumor tissue samples could not be traced. Finally, 771 (95%) of the available tissue samples were retrieved and 734 (90%) of these contained sufficient tumor material, making molecular analyses possible [10]. DNA isolation is described elsewhere [10]. Briefly, sections (5 μm) were cut from paraffin-embedded tumor tissue blocks and stained with haematoxylin and eosin (H&E) for histopathological examination. Five 20-μm sections of tumor tissue were used for DNA extraction. Tumor tissue was macrodissected from the normal colonic epithelium using the HE section as a reference. Proteinase K stock solution (20 mg/ml, obtained form Qiagen, St. Louis, MO, USA) and the Puregene® DNA Isolation Kit (Gentra Systems, Minneapolis, MN, USA) were used to extract genomic DNA from the macrodissected tumor tissue. The DNA concentration and purity were established spectrophotometrically at 260 and 280 nm [10].

Mutation analysis of the exon 1 fragment of the *KRAS* oncogene, spanning codons 8–29, was performed on archival colorectal adenocarcinoma specimens of 734 patients, using macrodissection, nested polymerase chain reaction (PCR) and direct sequencing of purified fragments. The detection limit was 5% mutated DNA. Duplicate experiments revealed a good reproducibility (88%) [10].

Exposure to tobacco smoke of all cancer cases and sub-cohort members was derived from the questionnaire. Exposure to smoke from cigarettes was characterized by eight smoking variables: smoking status (never, former and current), frequency, duration, pack years of smoking, age at first exposure, years since cessation, inhalation and filter usage. The never-smoker category was considered to be the reference for all variables. For categorical analyses, frequency was divided in the categories: less than 10, 10–20 and more than 20 cigarettes smoked per day. Duration of smoking was categorized as: less than 20, 20–40 and more than 40 years of cigarette smoking. The variable pack-years was divided in the categories: less than 9, 10–30 and more than 30 pack-years of cigarette smoking. Age at first exposure was categorized as: younger than 17, 17–21 and older than 21 years of age. Years since cessation was divided in the categories: less than 10, 10–30 and more than 30 years since cessation. Inhalation was categorized as yes or no. Filter usage was divided in filter- and non-filter-tipped cigarette use.

The dietary section of the questionnaire is a 150-item semi-quantative food-frequency questionnaire, which concentrated on habitual consumption of food and beverages during the year preceding the start of the study as well on lifestyle factors. Questionnaires were key-entered twice and automatically coded by the data-entry program. Data were checked for completeness, consistency, range and other response errors and corrected by means of an SPSS computer program. Questionnaires were excluded when incomplete or inconsistent. Criteria used for this selection were (a) 60 or more questionnaire items left blank or consumption of 35 or more food items less than once a month and/or (b) one or more item blocks (groups of items, for example beverages) left blank. Additional details are given elsewhere [16].

The distribution of the smoking variables as well as the distributions of the variables sex, family history of colorectal cancer (yes/no) and physical activity in leisure time (<30, 30–60, 60–90, >90 min/day), and the mean value of age at baseline (years), intake of energy (kJ/day), fat (g/day), dietary fiber (g/day), alcohol (g/day), coffee (ml/day), calcium (mg/day), vitamin A (mg/day), vitamin C (mg/day), vitamin E (mg/day), β-carotene (μg/day) and body mass index (BMI) (kg/m^2), were evaluated for sub-cohort members and colorectal cancer patients with wild-type or mutated *KRAS* gene tumors. Differences

in the mean values of the continuous variables between patients with wild-type or mutated *KRAS* gene tumors were tested with the Student's *t*-test or Mann–Whitney *U* test if the variables were not normally distributed. We used the χ^2 test to test for differences in the distributions of the categorical variables between patients with wild-type or mutated *KRAS* gene tumors. The analyses were conducted separately for colorectal cancer overall, for colorectal cancer cases with wild-type or mutated *KRAS* gene tumors, and for tumors with a specific C:G → T:A or G:C → T:A point mutation. Similar analyses were conducted for different sub-sites of the colorectal tract, i.e. proximal and distal colon cancer, rectosigmoid cancer and rectal cancer and the different molecular subgroups thereof. No differences were observed between any of the sub-sites, therefore only data of the analyses for colorectal cancer overall are presented.

Incident rate ratios (RR) and corresponding 95% confidence intervals (CI) for colorectal cancer cases with wild-type or mutated *KRAS* gene tumors were estimated according to cigarette smoking status (never, ex, current), smoking frequency (cigarettes/day), smoking duration (years), pack years, age when starting smoking (years), time since smoking cessation (years) and corresponding continuous variables compared with lifelong non-smokers using Cox proportional hazards regression models with the STATA statistical software package (intercooled STATA, Version 8.2). Standard errors were estimated using the robust Huber–White sandwich estimator to account for additional variance introduced by sampling from the cohort [17]. The proportional hazard assumption was tested using the scaled Schoenfeld residuals [18]. Tests for dose-response trends over the different categories of smoking variables were estimated by fitting the ordinal exposure variables as continuous terms and evaluated using the Wald test. Life-long non-smokers were excluded for estimation of the *p*-value for trend over the categories of age when started smoking and years since smoking cessation.

All potential confounders were separately tested in the models for overall colorectal cancer with wild-type or mutated *KRAS* gene tumors. Variables that showed a ≥10% influence on the RR for cancer or were found to contribute (p-value < 0.10) to any of the multivariate models (age at baseline, sex), by means of the Wald test were included in the multivariate analyses. Variables that were identified as confounders in the literature were also considered confounders. Finally age, sex, family history of colorectal cancer, BMI, alcohol and coffee consumption were considered as confounders and analyses were adjusted for these variables. Among all members and cases, questions on whether subjects drank coffee were left blank by 10 persons. They were considered to be non-drinkers. Forty-four subjects reported drinking coffee, but did not report how much. They were assumed to drink the mean amount of coffee consumed by the drinkers in the sub-cohort (534 ml/day). For 143 sub-cohort members and 24 colorectal cancer cases data on BMI were missing. Data on alcohol were missing for 117 sub-cohort members and 11 colorectal cancer cases. These members and cases were excluded from the analyses. The Pearson correlation coefficient between smoking duration and smoking frequency was strong ($r = 0.80$). Therefore, the variable smoking duration is not adjusted for frequency, and the variable smoking frequency is not adjusted for duration. Eventually, 648 colorectal cancer patients (367 men, 281 women) and 4083 sub-cohort members (2031 men, 2052 women) were included in the analyses.

Possible interaction by sex, alcohol, vitamin A, vitamin C, vitamin E and β-carotene was investigated by entering an interaction term in the model and assessing the significance of this term using the Wald test. No interactions were observed for any of the mentioned variables, and therefore results are shown combined for men and women and for the other variables. Nevertheless, since men and women differ considerably with respect to smoking habits, analyses for the risk of *KRAS* wild-type and mutated colorectal tumors according to the main smoking variables (smoking status, frequency and duration) are also presented separately for men and women. Finally, since ex-smokers, compared with never smokers, appeared to be at a higher risk for cancer in some subgroup analyses and because current smokers were never at increased risk, analyses were also conducted separately for ex- and current smokers. Specifically, analyses of the risk for *KRAS* wild-type and mutated colorectal tumors with frequency and duration of smoking are presented separately for the ex-smokers and current smokers, using never smokers as a reference. These extra analyses are not presented for cancers with specific mutations, since in each of these analyses at least one of the cells contained less than 10 cases, which hampers a sound interpretation of the results.

All *p*-values reported are for a two-sided test; *p*-values of less than 0.05 were considered to be statistically significant.

3. Results

Smoking habits and other baseline characteristics for sub-cohort members and colon, rectosigmoid and rectal cancer cases with wild-type or mutated *KRAS* gene tumors are shown in Table 1. Colon, rectosigmoid and rectal cancer cases were more often men, were older and more frequently reported a family history of colorectal cancer compared with the sub-cohort. Colon cancer cases with mutated *KRAS* tumors were significantly older, had higher daily intakes of vitamin E and more frequently reported smoking both cigarettes and cigars than colon cancer cases with a wild-type *KRAS* tumor, but the latter finding is based on few cases (i.e. 17 and 18, respectively). There were no statistically significant differences between colon cancer cases with a wild-type *KRAS* tumor and a mutated *KRAS* tumor in all other factors presented in Table 1. No statistically significant differences were observed between rectosigmoid cancer cases with wild-type *KRAS* tumors and mutated *KRAS* tumors or between rectal cancer cases with wild-type *KRAS* tumors and mutated *KRAS* tumors for smoking and other factors. Since smoking of cigars and pipe was rare (<1%) further data will only be presented for cigarette smoking.

Multivariate-adjusted RR for colorectal cancer overall, colorectal cancer with wild-type *KRAS* tumors and colorectal cancer with mutated *KRAS* tumors according to the smoking variables are presented in Table 2. The presented multivariate RRs are based on 648 colorectal cancer patients and 4083 sub-cohort members. Ex-smokers were at increased risk for overall colorectal cancer compared with never smokers (RR 1.22; 95% CI 0.97–1.53), but the association was not statistically significant. Current smokers were not at increased risk for overall colorectal cancer in comparison with never smokers (RR 0.81; 95% CI 0.61–1.05). There were no significant associations between smoking frequency, smoking duration, pack years, age at first exposure, years since cessation, inhalation or filter usage and the risk for colorectal cancer. Associations between ex-smokers, smoking frequency, inhalation and the risk for colorectal cancer with a wild-type *KRAS* gene appeared to be stronger than for the risk for colorectal cancer with a mutated *KRAS* gene, where these associations were absent. Ex-smokers were at increased risk for developing colorectal cancer with a wild-type *KRAS* gene when compared with never smokers (RR 1.26; 95% CI 0.96–1.66), although the association was not statistically significant. The highest category of smoking frequency (>20 cigarettes/day) and inhalation were associated with an increased risk for colorectal cancer with a wild-type *KRAS* gene, although not statistically significant (frequency: RR 1.24; 95% CI 0.90–1.71 and inhalation: RR 1.25; 95% CI 0.94–1.67). No associations were observed between the remaining smoking variables, i.e. smoking duration, pack years, age at first exposure, years since cessation, filter usage and the risk for colorectal cancer with wild-type *KRAS* gene tumors. Regarding mutated *KRAS* tumors, none of the smoking variables were associated with risk for this type of colorectal cancer.

Table 1
Baseline characteristics of subcohort members, colon, rectosigmoid and rectal cancer cases with wild-type or mutated *KRAS* gene tumors from the NLCS after 7.3 years of follow-up[a]

	Subcohort	Colon cancer			Rectosigmoid cancer			Rectal cancer		
		Wild-type *KRAS*	Mutated *KRAS*	*p*-Value[b]	Wild-type *KRAS*	Mutated *KRAS*	*p*-Value[b]	Wild-type *KRAS*	Mutated *KRAS*	*p*-Value[b]
N	4,083	288	140		42	28		87	63	
Gender (% male)	49.7	51.4	58.6	*0.162*	52.4	57.1	*0.695*	72.4	57.1	*0.051*
Age (years, mean (S.D.))	61.3 (4.2)	62.6 (4.1)	63.7 (3.9)	*0.015*	62.4 (3.9)	63.1 (4.1)	*0.380*	62.7 (4.0)	61.9 (3.9)	*0.202*
Family History of CRC (n (% yes))	235 (5.8)	37 (12.9)	14 (10.0)	*0.394*	4 (9.5)	0 (0)	*0.093*	9 (10.3)	8 (12.7)	*0.654*
BMI (kg/m^2, mean (S.D.))	25.0 (3.1)	25.5 (3.2)	25.7 (3.3)	*0.371*	25.6 (2.6)	25.6 (3.4)	*0.981*	24.9 (2.9)	25.5 (2.9)	*0.283*
Physical activity (*n*, (%))										
<30	825 (20.4)	56 (19.7)	32 (23.4)		6 (14.3)	4 (14.3)		17 (19.5)	16 (25.8)	
30–60	1,274 (31.5)	92 (32.3)	42 (30.7)		11 (26.2)	10 (35.7)		26 (29.9)	18 (29.0)	
60–90	850 (21.0)	56 (19.7)	32 (23.4)		10 (23.8)	9 (32.1)		17 (19.5)	14 (22.6)	
>90	1091 (27.0)	81 (28.4)	31 (22.6)	*0.481*	15 (35.7)	5 (17.9)	*0.421*	27 (31.0)	14 (22.6)	*0.625*
Energy intake (kJ/day, mean (S.D.))	8,072 (2,159)	7,982 (2,053)	80,734 (1,962)	*0.661*	77,078 (2,532)	8,009 (1,953)	*0.597*	8,404 (1,865)	8,375 (1,918)	*0.925*
Fat intake (g/day, mean (S.D.))	84.1 (27.5)	83.2 (26.2)	84.7 (26.0)	*0.567*	82.2 (35.3)	82.3 (22.9)	*0.986*	85.0 (23.1)	88.4 (27.1)	*0.412*
Alcohol consumption (g/day, mean (S.D.))	10.4 (14.4)	10.9 (15.4)	10.9 (14.4)	*0.965*	8.8 (9.0)	16.4 (16.1)	*0.106*	14.1 (17.9)	11.4 (12.3)	*0.648*
Coffee consumption (ml/day, mean (S.D.))	538.7 (270.0)	537.5 (251.0)	541.1 (279.2)	*0.919*	503.0 (236.7)	517.9 (191.6)	*0.652*	575.1 (260.0)	584.9 (265.3)	*0.966*
Calcium intake (mg/day, mean (S.D.))	926.5 (326.3)	929.6 (318.0)	910.5 (299.2)	*0.553*	892.6 (325.2)	828.5 (273.3)	*0.393*	944.4 (299.4)	866.3 (273.1)	*0.104*
Dietary fiber intake (g/day, mean (S.D.))	27.0 (8.1)	26.8 (7.6)	28.1 (8.7)	*0.093*	27.0 (8.9)	25.7 (7.2)	*0.523*	27.9 (7.9)	27.7 (8.0)	*0.891*
Vitamin A intake (mg/day, mean (S.D.))	1.0 (0.4)	0.9 (0.4)	1.0 (0.4)	*0.820*	1.2 (0.8)	0.9 (0.3)	*0.185*	1.0 (0.3)	1.0 (0.4)	*0.880*
Vitamin C intake (mg/day, mean (S.D.))	103.7 (43.6)	102.3 (44.4)	108.1 (45.2)	*0.208*	118.3 (47.2)	109.0 (52.5)	*0.441*	110.6 (43.5)	106.5 (48.7)	*0.586*
Vitamin E intake (mg/day, mean (S.D.))	13.4 (6.1)	13.3 (6.1)	14.8 (6.7)	*0.024*	13.5 (7.9)	13.8 (6.3)	*0.852*	14.1 (5.9)	14.3 (7.7)	*0.859*
β-carotene intake (μg/day, mean (S.D.))	2,961 (1,556)	2,878 (1,305)	3,022 (1,517)	*0.681*	3,399 (1,614)	3,062 (1,411)	*0.415*	2,948 (1,225)	2,935 (1,554)	*0.536*
Tobacco product (*n*, (%))										
Never smokers	1,375 (33.7)	97 (33.7)	50 (35.7)	*0.678*	9 (21.4)	3 (10.7)	*0.244*	20 (23.0)	19 (30.2)	*0.323*
Cigarette	2,076 (50.8)	137 (47.6)	61 (43.6)	*0.436*	24 (57.1)	17 (60.7)	*0.766*	50 (57.5)	29 (46.0)	*0.166*
Cigar	42 (1.0)	2 (0.7)	1 (0.7)	*0.982*	0 (0)	0 (0)	–	1 (1.2)	0 (0)	*0.393*
Pipe	8 (0.2)	1 (0.4)	0 (0)	*0.485*	0 (0)	0 (0)	–	1 (1.2)	0 (0)	*0.393*
Cigarette and pipe	86 (2.1)	2 (0.7)	4 (2.9)	*0.074*	1 (2.4)	0 (0)	*0.411*	2 (2.3)	3 (4.8)	*0.407*
Cigarette and cigar	268 (6.6)	18 (6.3)	17 (12.1)	*0.037*	3 (7.1)	6 (21.4)	*0.080*	8 (9.2)	8 (12.7)	*0.493*
Cigar and pipe	22 (0.5)	3 (1.0)	1 (0.7)	*0.741*	0 (0)	0 (0)	–	0 (0)	1 (1.6)	*0.238*
All three products	206 (5.1)	28 (9.7)	6 (4.3)	*0.051*	5 (11.9)	2 (7.1)	*0.515*	5 (5.8)	3 (4.8)	*0.791*

[a] Numbers are based on subcohort members and patients without data missing on family history of colorectal cancer, BMI, alcohol or coffee consumption.
[b] *p*-Value for comparison between patients with wild-type and mutated *KRAS* gene tumors.

Table 2
Adjusted[a] incidence rate ratios (RR) and corresponding 95% CI for colorectal cancer, colorectal cancer with wild-type and mutated *KRAS* gene tumors according to cigarette smoking features

Cigarette smoking variable	Personyears subcohort[b]	Colorectal cancer			Colorectal cancer–wild-type *KRAS*			Colorectal cancer–mutated *KRAS*		
		N[c]	RR	95% CI	*N*[c]	RR	95% CI	*N*[c]	RR	95% CI
Smoking status										
Never smoker	9,896	198	1.00	Reference	126	1.00	Reference	72	1.00	Reference
Ex-smoker	10,502	295	1.22	0.97–1.53	188	1.26	0.96–1.66	107	1.15	0.79–1.66
Current smoker	8,181	144	0.81	0.62–1.05	95	0.86	0.62–1.18	49	0.72	0.47–1.10
Frequency (cigarettes/day)										
<10	5,002	97	0.94	0.72–1.24	63	0.98	0.70–1.36	34	0.88	0.57–1.36
10–20	6,028	142	1.08	0.82–1.42	94	1.16	0.84–1.61	48	0.93	0.60–1.46
>20	6,605	171	1.16	0.89–1.52	111	1.24	0.90–1.71	60	1.02	0.66–1.58
p-Trend			0.23			0.14			0.88	
Duration (years)										
<20	3,288	69	1.07	0.79–1.47	48	1.19	0.82–1.73	21	0.87	0.52–1.45
20–40	8,636	219	1.15	0.91–1.46	136	1.16	0.87–1.55	83	1.13	0.78–1.65
>40	6,389	144	0.86	0.65–1.13	94	0.93	0.67–1.30	50	0.73	0.47–1.15
p-Trend			0.43			0.76			0.33	
Pack years										
<9	4,905	99	1.01	0.77–1.33	66	1.07	0.77–1.84	33	0.91	0.59–1.41
10–30	7,336	169	1.04	0.80–1.36	112	1.12	0.82–1.54	57	0.91	0.59–1.39
>30	5,141	138	1.12	0.84–1.49	87	1.16	0.82–1.64	51	1.03	0.64–1.64
p-Trend			0.47			0.37			0.95	
Age at first exposure (years)										
<17	7,170	185	1.18	0.89–1.56	116	1.18	0.85–1.66	69	1.17	0.74–1.84
17–21	7,310	167	1.08	0.84–1.40	105	1.08	0.79–1.48	62	1.08	0.71–1.63
>21	3,980	78	0.90	0.67–1.20	54	0.99	0.70–1.39	24	0.75	0.46–1.22
p-Trend			0.45			0.84			0.27	
Years since cessation (years)										
<10	11,830	252	0.96	0.76–1.22	165	1.02	0.77–1.35	87	0.86	0.59–1.26
10–30	5,873	168	1.24	0.96–1.61	104	1.24	0.91–1.70	64	1.23	0.82–1.86
>30	924	17	0.78	0.45–1.33	13	0.97	0.52–1.78	4	0.47	0.17–1.32
p-Trend			0.33			0.29			0.78	
Inhalation										
No	5,354	107	0.90	0.69–1.17	67	0.92	0.66–1.27	40	0.86	0.56–1.31
Yes	12,874	318	1.15	0.91–1.47	209	1.25	0.94–1.67	109	0.99	0.67–1.47
Filter usage										
Filter-tipped	5,043	103	1.04	0.79–1.36	66	1.05	0.76–1.46	37	1.01	0.66–1.54
Non-filter-tipped	8,892	230	1.07	0.82–1.40	148	1.12	0.82–1.53	82	1.00	0.65–1.53

[a] RRs are adjusted for age (years), sex, family history of colorectal cancer (yes/no), body mass index (kg/m^2), alcohol and coffee consumption (g/day).
[b] Personyears are estimated from the subcohort.
[c] Missing values of smoking characteristics gave rise to diminished personyears and number of colorectal cancer cases for the different smoking variables.

Table 3 presents the multivariate-adjusted RRs and corresponding 95% confidence intervals for colorectal cancer patients with G:C → T:A transversions ($n = 72$) and patients with G:C → A:T transitions ($n = 131$) in *KRAS* in their tumors. With regard to G:C → T:A transversions, smoking frequency was significantly and inversely associated with the risk for tumors with such mutations ($p = 0.03$). None of the other smoking variables were associated with the risk for this type of tumor, but the number of cases in some of the smoking categories is too small to adequately interpret the results. Regarding colorectal cancer with specific G:C → A:T transitions, a J-shaped association was observed with smoking frequency. The incidence rate ratio was significantly decreased for the lowest category of frequency (RR 0.44; 95% CI 0.21–0.94) and was non-significantly increased for the highest category (RR 1.43; 95% CI 0.83–2.45). There were no significant associations between risk for colorectal cancer with specific G:C → A:T transitions and smoking status, duration, pack years, age at first exposure, years since cessation, inhalation or filter usage.

The analyses described above were also conducted for colon, rectosigmoid and rectum cancer separately, and there were no apparent differences in associations with smoking variables between various sites of the colorectal tract, although in some subgroups the numbers of cases were too small to draw definite conclusions. Therefore, the data for the various sites are not shown and discussed any further.

In general, associations with the risk for wild-type *KRAS* colorectal tumors appeared stronger in men (Table 4) than in the

Table 3
Adjusted[a] incidence rate ratios (RR) and corresponding 95% CI for colorectal cancer with G:C>T:A transversions and G:C > A:T transitions

Cigarette smoking variable	Personyears subcohort[b]	Colorectal cancer with G:C > T:A transversions			Colorectal cancer with G:C > A:T transitions		
		N[c]	RR	95% CI	*N*	RR	95% CI
Smoking status							
Never smoker	9,896	25	1.00	Reference	40	1.00	Reference
Ex-smoker	10,502	28	0.95	0.49–1.85	63	1.16	0.72–1.86
Current smoker	8,181	19	0.84	0.43–1.66	28	0.70	0.41–1.21
Frequency (cigarettes/day)							
<10	5,002	19	1.35	0.72–2.56	9	0.44	0.21–0.94
10–20	6,028	12	0.59	0.27–1.31	29	1.11	0.62–2.00
>20	6,605	12	0.50	0.23–1.10	43	1.43	0.83–2.45
p-Trend			0.03			0.08	
Duration (years)							
<20	3,288	8	0.98	0.44–2.19	12	0.89	0.45–1.76
20–40	8,636	20	0.81	0.40–1.63	51	1.23	0.76–1.96
>40	6,389	17	0.71	0.34–1.50	28	0.70	0.39–1.26
p-Trend			0.35			0.40	
Pack years							
<9	4,905	14	1.09	0.56–2.15	13	0.68	0.35–1.29
10–30	7,336	15	0.63	0.29–1.36	34	1.04	0.60–1.81
>30	5,141	13	0.68	0.31–1.50	34	1.29	0.71–2.32
p-Trend			0.22			0.32	
Age at first exposure (years)							
<17	7,170	24	1.40	0.62–3.20	37	1.03	0.58–1.82
17–21	7,310	16	0.93	0.44–1.99	39	1.14	0.68–1.93
>21	3,980	6	0.56	0.22–1.40	15	0.80	0.43–1.48
p-Trend			0.13			0.71	
Years since cessation (years)							
<10	1,1830	28	0.82	0.43–1.58	50	0.85	0.53–1.38
10–30	5,873	17	1.02	0.48–2.13	38	1.28	0.75–2.18
>30	924	1	0.36	0.05–2.66	3	0.63	0.19–2.06
p-Trend			0.65			0.58	
Inhalation							
No	5,354	16	1.01	0.51–1.99	20	0.76	0.43–1.32
Yes	12,874	29	0.77	0.39–1.52	66	1.05	0.63–1.75
Filter usage							
Filter-tipped	5,043	16	1.19	0.62–2.29	19	0.92	0.52–1.64
Non-filter-tipped	8,892	20	0.53	0.26–1.07	50	1.21	0.70–2.10

[a] RRs are adjusted for age (years), sex, family history of colorectal cancer (yes/no), body mass index (kg/m^2), alcohol and coffee consumption (g/day).
[b] Personyears are estimated from the subcohort.
[c] Missing values of smoking characteristics gave rise to diminished personyears and number of colorectal cancer cases for the different smoking variables.

total group as presented in Table 2. Associations with smoking variables were absent for *KRAS*-mutated colorectal tumors in men and for both end points in women (Table 4). In men, the risk of *KRAS* wild-type colorectal tumors was increased significantly in ex-smokers compared with never smokers (RR 1.79, 95% CI 1.00–3.20), whereas for current smokers no significantly increased risk was observed compared with never smokers (RR 1.23, 95% CI 0.66–2.31). There was a significant trend for increased *KRAS* wild-type colorectal cancer risk with increasing frequency of cigarette smoking in men (*p*-trend = 0.02). For men smoking more than 20 cigarettes/day, compared with never smokers, the RR was 1.91, which was statistically significant (95% CI 1.03–3.52). For men inhaling cigarette smoke versus never smokers, the RR for *KRAS* wild-type tumors was also elevated, although not statistically significantly (RR 1.69, 95% CI 0.94–3.04, not shown in the table).

For ex-smokers, both frequency and duration of smoking were associated with an increased risk for wild-type *KRAS* colorectal tumors, when compared with never smokers, although this was not statistically significant (Table 5). Smoking more than 20 cigarettes/day versus never smoking was associated with a 35 percent increased risk of *KRAS* wild-type tumors (95% CI 0.92–1.99 and *p*-trend = 0.09). Smoking cigarettes for more than 40 years was associated with a 44% increased risk of *KRAS* wild-type tumors (95% CI 0.89–2.33, *p*-trend = 0.07). Although the risk for mutated *KRAS* tumors was also elevated, although not statistically significantly, in ex-smokers who smoked more than 20 cigarettes/day versus never smokers, there was no clear trend (RR 1.40, 95% CI 0.84–2.33, *p*-trend = 0.22). In current

Table 4
Sex-specific adjusted[a] incidence rate ratios (RR) and corresponding 95% CI for colorectal cancer with wild-type and mutated *KRAS* gene tumors according to cigarette smoking status, frequency and duration

Cigarette smoking variable	Personyears subcohort[b]	Colorectal cancer–wild-type *KRAS*			Colorectal cancer–mutated *KRAS*		
		N[c]	RR	95% CI	*N*[c]	RR	95% CI
		Men					
Smoking status							
Never smoker	1366	14	1.00	Reference	11	1.00	Reference
Ex-smoker	7444	147	1.79	1.00–3.20	85	1.20	0.61–2.33
Current smoker	5036	64	1.23	0.66–2.31	35	0.78	0.39–1.58
Frequency (cigarettes/day)							
<10	2294	34	1.33	0.69–2.57	21	1.02	0.48–2.19
10–20	4236	66	1.48	0.80–2.76	35	0.93	0.46–1.90
>20	5148	97	1.91	1.03–3.52	53	1.17	0.58–2.36
p-Trend			0.02			0.57	
Duration (years)							
<20	1758	29	1.77	0.90–3.48	15	1.15	0.52–2.58
20–40	5545	100	1.72	0.95–3.12	65	1.31	0.67–2.58
>40	4971	78	1.39	0.74–2.59	40	0.78	0.38–1.59
p-Trend			0.82			0.20	
		Women					
Smoking status							
Never smoker	8530	112	1.00	Reference	61	1.00	Reference
Ex-smoker	3058	41	1.09	0.74–1.62	22	1.10	0.67–1.82
Current smoker	3146	31	0.75	0.48–1.17	14	0.67	0.35–1.28
Frequency (cigarettes/day)							
<10	2708	29	0.89	0.57–1.41	13	0.75	0.40–1.40
10–20	1791	28	1.26	0.80–1.97	13	1.18	0.63–2.19
>20	1457	14	0.69	0.38–1.25	7	0.68	0.28–1.64
p-Trend			0.60			0.57	
Duration (years)							
<20	1530	19	1.04	0.61–1.76	6	0.63	0.26–1.49
20–40	3091	36	0.96	0.63–1.46	18	0.96	0.55–1.68
>40	1419	16	0.79	0.46–1.36	10	0.94	0.46–1.91
p-Trend			0.48			0.79	

[a] RRs are adjusted for age (years), family history of colorectal cancer (yes/no), body mass index (kg/m^2), alcohol and coffee consumption (g/day).
[b] Personyears are estimated from the subcohort.
[c] Missing values of smoking characteristics gave rise to diminished personyears and number of colorectal cancer cases for the different smoking variables.

smokers, there was no increased risk for wild-type or mutated *KRAS* tumors according to frequency or duration of smoking. For mutated *KRAS* tumors, a slight protective effect was even observed in current smokers with frequency (p-trend = 0.06) and duration (p-trend = 0.05) of smoking. However, we have to acknowledge that the number of cases were low in the current smoking group of cases with mutated *KRAS* tumors. Specifically, since people generally started smoking at a very early age, the number of current smokers who smoked less than 10 years was rare.

4. Discussion

In the Netherlands Cohort Study on diet and cancer (NLCS) we observed that current smoking was not associated with the risk for overall colorectal cancer, colorectal cancer with wild-type *KRAS* tumors or colorectal cancer with mutated *KRAS* tumors. Former smoking, frequency of cigarette smoking and inhalation were non-significantly associated with increased risk of colorectal cancer overall and with colorectal cancer with a wild-type *KRAS* gene compared to never smoking, whereas associations were not apparent with respect to the risk for colorectal cancer with mutated *KRAS* tumors. The associations with wild-type *KRAS* tumors appeared strongest for men and ex-smokers and were not apparent for women and current smokers. Furthermore, no clear associations were observed between smoking and colorectal cancer with specific G:C → T:A transversions or G:C → A:T transitions in *KRAS*. In contrast to what we expected, smoking was not associated with the risk for colorectal cancer with *KRAS* oncogene mutations.

Few studies have looked at cigarette smoking and colorectal cancer while assessing *KRAS* mutation status. A summary of the studies is given in Table 6. Regarding risk for *KRAS*-mutated tumors, none of the four studies showed a significant association between cigarette smoking and risk for colon carcinomas or colorectal adenomas with mutated *KRAS* [19–22]. This is in line with our observations that none of the investigated cigarette-smoking variables are associated with the risk for col-

Table 5
Adjusted[a] incidence rate ratios (RR) and corresponding 95% CI for colorectal cancer with wild-type and mutated *KRAS* gene tumors according to cigarette smoking frequency and duration for ex-smokers and current smokers separately

Cigarette smoking variable	Personyears subcohort[b]	Colorectal cancer—wild-type *KRAS*			Colorectal cancer—mutated *KRAS*		
		N[c]	RR	95% CI	*N*[c]	RR	95% CI
		Ex-smokers[d]					
Frequency (cigarettes/day)							
Never smoker	9896	126	1.00	Reference	72	1.00	Reference
<10	3172	45	1.12	0.77–1.62	25	1.03	0.63–1.68
10–20	3130	62	1.41	0.96–2.08	29	1.07	0.61–1.85
>20	3858	73	1.35	0.92–1.99	48	1.40	0.84–2.33
p-Trend			0.09			0.22	
Duration (years)							
Never smoker	9896	126	1.00	Reference	72	1.00	Reference
<20	2843	42	1.25	0.84–1.85	15	0.76	0.42–1.38
20–40	5946	108	1.32	0.96–1.83	72	1.40	0.91–2.16
>40	1409	35	1.44	0.89–2.33	19	1.13	0.59–2.15
p-Trend			0.07			0.23	
		Current smokers[e]					
Frequency (cigarettes/day)							
Never smoker	9896	126	1.00	Reference	72	1.00	Reference
<10	1830	18	0.80	0.47–1.38	9	0.64	0.31–1.33
10–20	2898	32	0.94	0.59–1.51	19	0.82	0.45–1.49
>20	2748	38	1.09	0.71–1.68	12	0.48	0.24–0.98
p-Trend			0.80			0.06	
Duration (years)							
Never smoker	9896	126	1.00	Reference	72	1.00	Reference
<20	445	6	1.03	0.42–2.53	6	1.79	0.73–4.39
20–40	2690	28	0.95	0.59–1.51	11	0.61	0.31–1.20
>40	4981	59	0.85	0.58–1.26	31	0.61	0.35–1.06
p-Trend			0.44			0.05	

[a] RRs are adjusted for age (years), sex, family history of colorectal cancer (yes/no), body mass index (kg/m^2), alcohol and coffee consumption (g/day).
[b] Personyears are estimated from the subcohort.
[c] Missing values of smoking characteristics gave rise to diminished personyears and number of colorectal cancer cases for the different smoking variables.
[d] In analyses for ex-smokers all current smokers were deleted from the analyses, and never smokers form the reference category.
[e] In analyses for current smokers all ex-smokers were deleted from the analyses, and never smokers form the reference category.

orectal carcinomas with mutated *KRAS*. However, in contrast to our study, the reported risks in the other studies in Table 6 are slightly elevated, although they never reach statistical significance.

We expected smoking variables to be associated with tumors harboring specific point mutations in *KRAS* since genotoxic compounds of cigarette smoke have been shown to induce specific G:C → A:T and G:C → T:A mutations in *RAS* oncogenes in rodents. The only finding from our study that fits this hypothesis is the non-significantly elevated risk for tumors with G:C > A:T transitions with smoking more than 20 cigarettes a day versus never smoking. However, the overall association between smoking frequency and tumors with G:C > A:T transitions had a U-shape, and a significant inverse trend with smoking frequency was observed for tumors with G:C → T:A transversions. Finally, we expected an association with long-term smoking, thus smoking duration, but this was not observed. Overall this leaves us to conclude that there is no clear association between smoking variables and tumors with specific point mutations in *KRAS*. This is in line with the findings from Slattery's large case–control study [19] in which no associations between smoking and risks for G:C → A:T transitions or G:C → T:A transversions were observed when smoking ≥20 cigarettes/day was compared with not smoking (transitions OR 0.8, 95% CI 0.6–1.2, transversions OR 0.9, 95% CI 0.7–1.3). Diergaarde et al. [20] observed a positive association, although not significant, between cigarette smoking and tumors with G:C → T:A transversion mutations in *KRAS* (*KRAS* transversions vs. controls; OR 2.2, 95% CI 0.7–6.3). This association was not found between cigarette smoking and G:C → A:T transitions in *KRAS* (*KRAS* transitions vs. controls; OR 1.0, 95% CI 0.4–2.5) [20].

Surprisingly, the most consistent observation seems to be an association between cigarette smoking and wild-type *KRAS* colorectal tumors [19,21]. Two out of the four other studies summarized in Table 6 also suggest that associations between cigarette smoking and colorectal tumors are more pronounced with wild-type *KRAS* tumors. In line with our results, Slattery et al.[19] observed an increased risk for having a wild-type *KRAS* colon tumor when smoking ≥20 cigarettes/day (wild-type *KRAS* vs. controls; OR 1.3, 95% CI 1.1–1.6), compared with never smoking. Wark et al. [21] reported that both current and former smokers, compared with never smokers, are at increased risk for wild-type *KRAS* colorectal adenomas, but not mutated *KRAS* adenomas, in their case–control study (wild-type *KRAS*

Table 6
Summary of studies of cigarette smoking and colorectal cancer

Author	Reference	Design	# Cases/controls	Outcome	OR (95% CI)
Slattery et al.	[19]	Case–control study	Mutated *KRAS*: 453 Wild-type *KRAS*: 968 Controls: 2404	Colon carcinomas	>20 cigarettes/day vs. none cigarettes/day: Mutated *KRAS* vs. controls: 1.2 (0.9–1.5) Wild-type *KRAS* vs. controls: 1.3 (1.1–1.6) *KRAS* transversion vs. controls: 0.9 (0.7–1.3) *KRAS* transitions vs. controls: 0.8 (0.6–1.2)
Diergaarde et al.	[20]	Case–control study	Mutated *KRAS*: 64 Wild-type *KRAS*: 112 Controls: 249 *KRAS* transversions: 31 *KRAS* transitions: 33	Colon carcinomas	Ever smokers vs. never smokers: Mutated *KRAS* vs. controls: 1.4 (0.7–2.8) Wild-type *KRAS* vs. controls: 0.8 (0.5–1.4) *KRAS* transversion vs. controls: 2.2 (0.7–6.3) *KRAS* transitions vs. controls: 1.0 (0.4–2.5)
Wark et al.	[21]	Case–control study	Mutated *KRAS*: 81 Wild-type *KRAS*: 453 Controls: 709	Colorectal adenomas	Former smokers vs. never smokers: Mutated *KRAS* vs. controls: 1.20 (0.70–2.08) Wild-type *KRAS* vs. controls: 1.45 (1.08–1.96) Current smokers vs. never smokers: Mutated *KRAS* vs. controls: 1.27 (0.66–2.42) Wild-type *KRAS* vs. controls: 2.04 (1.47–2.83)
Martinez et al.	[22]	Cross-sectional study	Mutated *KRAS*: 120 Wild-type *KRAS*: 558	Colorectal adenomas	Past smokers vs. never smokers: Mutated *KRAS* vs. wild-type *KRAS*: 1.07 (0.67–1.70) Current smokers vs. never smokers: Mutated *KRAS* vs. wild-type *KRAS*: 1.17 (0.61–2.25)
Present study		Cohort study	Mutated *KRAS*: 81 Wild-type *KRAS*: 453 Subcohort: 4083	Colorectal cancer	Former smokers vs. never smokers: Mutated *KRAS* vs. never smokers: 1.15 (0.79–1.66) Wild type *KRAS* vs. never smokers: 1.26 (0.96–1.66) Current smokers vs. never smokers: Mutated *KRAS* vs. never smokers: 0.72 (0.47–1.10) Wild type *KRAS* vs. never smokers: 0.86 (0.62–1.18)

vs. controls; OR 2.04, 95% CI 1.47–2.83 and OR 1.45, 95% CI 1.08–1.96, for current and former smokers, respectively, compared with never smokers). In another case–control study on cigarette smoking and sporadic colon carcinomas by Diergaarde et al. [20], ever-smokers were not at increased risk for having tumors with wild-type *KRAS* (wild-type *KRAS* vs. controls; OR 0.8, 95% CI 0.5–1.4).

Our finding that the association of smoking variables with wild-type *KRAS* tumors was strongest for men and not apparent for women was also observed by Slattery et al. [19]. None of the other studies reported sex-specific associations [20–22]. In the present study, women smoked considerably less than men, leaving relatively small numbers of female cases in different subgroups of smoking variables (see Table 4). This may have influenced the results, but we should also note that there was no significant effect modification by gender in this study. However, it is worthwhile to further explore the strength of the association between smoking and *KRAS* wild-type colorectal cancer in women in future studies.

Our finding that associations between smoking frequency and duration and wild-type *KRAS* tumors was present in ex-smokers but not current smokers, all compared with never smokers, is puzzling. Others have not reported such differences [19–22]. In our population, most people who ever smoked were ex-smokers. Many of these ex-smokers had smoked at a high frequency and for a long time. Therefore, their lifetime exposure to smoking was still considerable and may have contributed to the increased cancer risk. The lack of association among the current smokers may reflect that this is a group of subjects that did not quit smoking because of smoking related ill-health and may therefore be less sensitive to the effects of smoking on colorectal cancer than the ex-smokers. However, it should be noted that the associations of the ex-smoking group were not statistically significant and that further research is warranted to tease out potential differential effects of ex- and current smokers on the risk for colorectal cancer, especially wild-type *KRAS* tumors.

Many years are needed to develop colorectal cancer after initiation. In this period, carcinogens from cigarette smoke cause irreversible genetic damage in the colorectal mucosa. Giovannucci et al. [23,24] suggested that the long lag time between exposure to cigarette smoke and occurrence of colorectal cancer is a possible explanation for smoking being a risk for colorectal adenomas but not for colorectal cancer. In the present study, 84% of the smokers had smoked for 20 years or more, 33% had smoked for 40 years or more and 43% started before the age of 19. These figures are high enough to assume that smoking could have caused colorectal cancer within the timeframe of this cohort study.

Our results indicate that smoking is not associated with the risk for colorectal cancer with *KRAS* oncogene mutations. However, smoking may be an early event in the development of colorectal cancers that arise through other underlying genetic pathways, such as mutations in the adenomatous polyposis coli (APC) tumor suppressor gene, P53 over-expression or absence

of hMLH1 expression [20,25–27]. Recently published results from the large case–control study discussed previously [19] suggest that smoking is related to CpG island methylator phenotype (CIMP) and V600E BRAF mutations in colon cancer, rather than with microsatellite-unstable cancer [28]. This could explain our results with regard to *KRAS* wild-type tumors, since *KRAS* mutations are rare in tumors with CIMP and/or BRAF mutations [29].

In contrast to case–control studies, the smoking habits of all subjects in this study were reported before the disease was diagnosed. Information bias, i.e. changes in smoking habits or change in report of smoking habits is therefore almost completely avoided in a prospective cohort study like this one. A change in smoking habits of subjects with pre-clinical colorectal cancer at the time of completing the baseline questionnaire remains possible. The completeness of follow-up together with the prospective nature of a cohort study reduced the potential for selection bias to a minimum. Underreporting of smoking habits because of social desirability remains possible. This could lead to non-differential misclassification of smoking habits. In this study, smoking characteristics are based on active smoking habits. Unfortunately, the effect of passive smoking is therefore not estimated. This may have attenuated the associations observed.

In conclusion, this study indicates that smoking is not associated with the risk for colorectal cancer with specific mutations in the *KRAS* gene. The study suggests that smoking may be associated with the risk for a specific molecular subgroup of colorectal cancer characterized by wild-type *KRAS* tumors. For an ex-smoker, smoking frequency and inhalation seemed to be associated with an increased risk for colorectal cancer with wild-type *KRAS* gene tumors, especially in men. Future studies should tease out whether there are other molecular characteristics of the *KRAS* wild-type tumors that may better explain the association with cigarette smoking.

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